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Effects of activated charcoal on binding *E. coli* O157:H7 and *Salmonella typhimurium* in sheep

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Abstract

Escherichia coli (*E. coli*) O157:H7 and *Salmonella typhimurium* have been implicated in food borne illness caused by contaminated milk and meat products. Activated charcoal (AC) was used to adsorb *E. coli* O157:H7 and *S. typhimurium* in the sheep gastrointestinal tract. Ewes were infected with either *E. coli* O157:H7 or *S. typhimurium* and were dosed with 10mg/ml AC slurry. AC had no effect on binding either organism in the rumen, cecum and rectum.

Keywords: *E. coli* O157:H7; *Salmonella typhimurium*; Sheep; Activated charcoal

1. Introduction

Estimated food borne infections by contaminated meat and produce are 76 million/year in the US (CDC, 2000). *Escherichia coli* (*E. coli*) O157:H7 and *Salmonella enteritidis* are two of the four microbes of concern in food production recognized by the Center for Disease Control and Prevention. Primarily, enterohemorrhagic *E. coli* (EHEC) and *Salmonella* spp., are common contaminants of meat products causing gastrointestinal infections. It is necessary to minimize the occurrence of food borne illness.

Activated charcoal (AC) is recognized as an adsorbent of drugs and other toxic substances from the gastrointestinal tract (GI) of man (Levy, 1982). AC helps to clear drugs that have already been absorbed and circulate in the system (Levy, 1982). AC effectively adsorb pesticides, environmental hydrocarbons, pharmaceutical agents, mycotoxins, phytotoxins, feed additives, antibacterials and most bacterial toxins (Buck and Bratich, 1986). AC binds its positively charged

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molecular surfaces with negatively charged molecular surfaces of toxins (Edwards and McCredie, 1967).

AC successfully treated a variety of toxicity problems in ruminants (Buck and Bratich, 1986; Bisson et al., 2001; Banner et al., 2000; Poage et al., 2000) and bacterial toxins (Buck and Bratich, 1986). AC is useful in the removal of *E. coli* O157:H7 organism and toxin, both in vitro and in vivo (Naka et al., 2001; Marks et al., 1998; Pegues et al., 1979).

Naka et al. (2001) reported significant reductions in *E. coli* O157:H7 concentrations (from 5.33×10^6 to 0.80×10^3 within 5 min) on feeding 5 mg/ml of AC. Initial concentration was reduced below detectable levels with 10 mg/ml.

Whether AC is effective in absorbing *E. coli* O157:H7 and *Salmonella typhimurium* in the sheep GI is reported.

2. Materials and methods

2.1. Animals and experimental design

Mature Rambouillet ewes ($n = 16$; approximately 68 kg in weight), were randomly assigned to Study 1 (*E. coli*; $n = 8$) or Study 2 (*Salmonella*; $n = 8$) and treated under approved animal care protocol.

2.2. E. coli study

On day 1, ewes ($n = 8$) were dosed via oral gavage with *E. coli* O157:H7 (ATCC 43895; 4.1×10^8 CFU/ml) at 12 ml/ewe. The inoculum was plated on MacConkey agar supplemented with novobiocin and nalidixic acid (Mac NN; 1–9 dilutions in 10-fold increments; Fisher Scientific, Houston, TX) to determine the challenge dose. On day 2, fecal samples were collected aseptically from recent droppings in 50 ml sterile Falcon tubes (2 tubes/pen) and 1 g placed in 9 ml of sterile phosphate buffered saline (PBS; Difco, Detroit, MI) and vortexed. Serial dilutions (10-fold) were prepared from the homogenate and plated on Mac NN (1–6 dilutions) with an incubation time of 24 ± 3 h at 39 °C to determine the concentration of the inoculated strain. Typical colonies appeared clear to opaque. On day 3, fecal samples were again collected and plated as described.

Activated charcoal solution was prepared on the basis that ruminal volume of an 82 kg sheep would be approximately 7.7 l (Hungate, 1966). A 10 mg/ml of AC (DARCO-KB, Norit Americas Inc., Atlanta, GA) was used as it has 100% binding effect on *E. coli* (Naka et al., 2001). Treatment ewes ($n = 4$) were dosed via oral gavage based on the following equations:

$$10\,\text{mg}/1\,\text{ml} = 1000\,\text{ml}/1\,\text{l} = 77,000\,\text{mg} = 77\,\text{g/hd}$$

$$0.208\,\text{g}/1\,\text{ml} = 77\,\text{g}/X\,\text{ml} = 370\,\text{ml/hd}$$

AC was suspended in reverse osmosis water at a concentration slightly below saturation (0.208 g/ml). On day 4, ewes ($n = 8$) were euthanized and tissue and content samples were taken from the rumen, cecum and rectum. Tissue samples were enriched in GN Hajne broth supplemented with novobiocin and nalidixic acid (GN Hajne NN; Difco, Detroit, MI) for 24 h at 39 °C. Content samples were diluted in PBS and plated on MacNN and MacConkey agar (Mac Plain; 2–8 dilutions; Difco, Detroit, MI) and incubated as described to determine generic coliforms. Typical colonies appeared clear to opaque. On day 5, the tissue samples enriched in GN Hajne NN were streaked on MacNN plates to confirm that *E. coli* O157:H7.

2.3. S. typhimurium study

Ewes ($n = 8$) were allowed free access to water and on day 1, ewes ($n = 8$) were dosed with *S. typhimurium* (ATCC 13311; 3×10^9 CFU/ml) at 12 ml/ewe. Fecal samples from each pen were collected, samples were prepared and diluted as previously described. Inoculum was plated on brilliant green agar supplemented with novobiocin and nalidixic acid (BGA NN; Difco, Detroit, MI) to determine the challenge dose. On day 2, fecal samples were collected and diluted as described and plated on BGA NN (1–4 dilutions) and incubated 24 ± 3 h at 39 °C. Typical colonies appeared pink or white surrounded by a zone of red. On day 3, fecal samples were collected, prepared, diluted, plated and incubated as described. Fecal samples were also enriched in Tetrathionate broth (TET broth; 20 ml broth with 400 μl iodine; Difco, Detroit, MI) and incubated for $24\,\text{h} \pm 3$ at 41 °C. Treatment ewes ($n = 4$) were dosed with AC (375 ml/ewe) and on day 4, ewes ($n = 8$) were euthanized. Tissue and content samples were taken from the rumen, cecum and rectum. The tissue samples were enriched in TET Broth and content samples were diluted in PBS and plated on BGA NN and Mac Plain plates. On day 5, tissue samples were

enriched in TET Broth and streaked on BGA NN plates to confirm the presence of *S. typhimurium*.

2.4. Statistical analysis

Effects of activated charcoal on *E. coli* O157:H7 and *S. typhimurium* adsorption in the sheep gastrointestinal tract was examined. For each study, treatment effects were compared utilizing the *T*-test function of SAS (SAS Inst. Inc., Carey, NC) with four experimental units for each treatment, using a predetermined alpha level of 0.05.

3. Results and discussion

3.1. E. coli study

Initial dose (8.61 $\log_{10}$ CFU/g) was plated on Mac NN and Mac Plain based on fecal, cecal and rumen samples. AC was administered to four animals (1–4) and 5–8 were the controls. Fecal samples indicated AC did not effectively bind *E. coli* O157:H7 compared to the control when plated on Mac NN ($P > 0.05$; 3.25 and 2.89 $\log_{10}$ CFU/g, respectively) and similar results were seen on Mac Plain plates (5.59 and 6.05 $\log_{10}$ CFU/g, respectively). Cecal samples showed similar results on Mac NN and Mac Plain ($P > 0.05$; 3.52 and 3.77 $\log_{10}$ CFU/g; 6.26 and 6.21 $\log_{10}$ CFU/g, respectively). Rumen samples also indicated no treatment differences on Mac NN and Mac Plain plates ($P > 0.05$; 2.45 and 2.30 $\log_{10}$ CFU/g; 4.77 and 4.55 $\log_{10}$ CFU/g, respectively). Naka et al. (2001) though found within 5 min, *E. coli* O157:H7 cellular concentrations of 5.33×10^6 were reduced to 0.80×10^3 with 5 mg/ml of AC, and to below detectable levels with 10 mg/ml. The current study in sheep, similar results were not found. Contents of the ruminant GI tract may interfere with the binding capabilities of the activated charcoal. Hayden and Comstock (1975) reported AC effectively bound drugs toxins in the GI tract of man by making it unavailable for reabsorption.

Buck and Bratich (1986) noted AC effectively adsorbs numerous toxins and drugs, however further research may be conducted to make AC a more effective adsorbent. Hayden and Comstock (1975) indicated AC's binding ability was approximately three times more effective in an acid solution than in a neutral solution. Further investigations may evaluate effectiveness of AC delivered in solutions of varying pH to detect if pH level is one of the reasons the organisms were not bound.

3.2. S. typhimurium study

Initial dose (9.00 $\log_{10}$ CFU/g) of *S. typhimurium* as plated on BGA NN and Mac Plain based on fecal, cecal and rumen samples. AC was administered to four animals (1–4) and not to the control animals (5–8). Fecal samples indicated AC did not effectively bind *S. typhimurium* compared to the control when plated on BGA NN ($P > 0.05$; 3.31 and 4.10 $\log_{10}$ CFU/g, respectively). Similar results were seen on Mac Plain plates (7.02 and 6.51 $\log_{10}$ CFU/g, respectively). BGA NN and Mac Plain plates indicated AC was not effective ($P > 0.05$) at binding the target organism in the cecum (4.09 and 4.98 $\log_{10}$ CFU/g; 6.92 and 6.53 $\log_{10}$ CFU/g, respectively). Rumen samples also indicated no effect ($P > 0.05$; 3.87 and 3.36 $\log_{10}$ CFU/g; 4.92 and 4.88 $\log_{10}$ CFU/g, respectively).

Attempt to utilize AC to bind pathogenic microorganisms (*E. coli* O157:H7 and *S. typhimurium*) was not successful in the present study. Buck and Bratich (1986) noted the effectiveness of AC to reduce GI infections caused by bacterial toxins in humans. They also noted that rapid administration of AC to prevent toxicosis and death is a suitable practice, which may have posed a problem in this study. It is possible that the time delay between inoculation with the target organism and dosing of AC inhibited the binding capabilities of the AC. Additional research could possibly explain the difference between the effectiveness of the AC in vitro and in situ by investigating various time increments between inoculation and the administering of AC. These include pre- and post-contamination methods to reduce *E. coli* and *Salmonella* contamination in animal products. Pre-contamination methods include feeding of hay, feed withdrawal, dosing with sodium chlorate and probiotic use. Diez-Gonzalez et al. (1998) reported feeding cattle hay before slaughter lowered levels of *E. coli* compared to feeding grain. Feed withdrawal time has also been reported to reduce the incidence of *Salmonella* spp. in pork carcasses (Miller et al., 1997). Callaway et al. (2002) found

sodium chlorate reduced the counts of *E. coli* O157:H7 throughout the GI tract in cattle. Sodium chlorate reduces the levels of *S. typhimurium* DT104 to below detectable levels in vitro (Anderson et al., 2000). Use of probiotic has also helped to reduce *E. coli* O157:H7 up to 50% in some cases (Brashears and Galyean, 2002).

Post-contamination methods include steam vacuum systems, application of edible films of potassium sorbate and lactic acid, acetic acid treatments, acidified sodium chlorite spray and electrical stimulation. Steam vacuum systems have been reported to lower levels of *E. coli* O157:H7 from 5 log to 1 log CFU/cm^2 (Dorsa et al., 1996). The application of an edible cornstarch mixed with potassium sorbate and lactic acid to poultry carcasses has inhibited *E. coli* O157:H7 growth (Baron, 1993). Acetic acid spray treatments have decreased *Salmonella* by 67% on pork cheek meat (Frederick et al., 1994). It has also been reported that the use of an acidified sodium chlorite system will reduce the levels of *E. coli* and *Salmonella* in commercially processed poultry (Kemp et al., 2001). Electrical stimulation has been reported to reduce the counts of *S. typhimurium* by 82% with a voltage level of 1200 V/2.5 cm (Bawcom et al., 1995).

4. Conclusion

Activated charcoal did not bind *E. coli* O157:H7 or *S. typhimurium* cells in the GI tract of sheep as reported in in vitro studies (Naka et al., 2001). However, the effectiveness of AC in binding the toxins of *E. coli* O157:H7 and *S. typhimurium* in vivo needs further investigation.

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